



Near-future temperature reduces Mg/Ca ratios in the major skeletal components of the common subtropical sea urchin *Lytechinus variegatus*

A. Duquette^a, Y.K. Vohra^b, J.B. McClintock^{a,*}, R.A. Angus^a

^a Department of Biology, University of Alabama at Birmingham, Birmingham, AL 35294, USA

^b Department of Physics, University of Alabama at Birmingham, Birmingham, AL 35294, USA

ARTICLE INFO

Keywords:

Magnesium calcite
Echinoid
Skeleton
Temperature
Climate change
Ocean acidification
Gulf of Mexico

ABSTRACT

Shallow-water marine invertebrates living near the upper limits of their thermal tolerances are uniquely susceptible to climate warming. This is particularly the case for species that occur in geographic regions where larval dispersal or adult migration to cooler waters is not an option. This scenario aptly describes populations of the seagrass-associated sea urchin *Lytechinus variegatus* inhabiting the northern Gulf of Mexico where northward movement is restricted by a coastline. In the present study, we exposed juvenile *L. variegatus* to chronic ambient (26 °C) and predicted near-future (30 °C) temperature treatments for 90 days. X-ray Diffraction (XRD) was employed to determine the Mg/Ca ratios of the spines, test, and Aristotle's lantern of individuals sub-sampled at 30, 60, and 90 days. We found that while individuals grew at similar rates, Mg/Ca ratios in all skeletal components were overall significantly lower in individuals held at 30 °C than at 26 °C.

1. Introduction

The current rate of increase of global atmospheric CO₂ is approximately an order of magnitude greater than that estimated to have occurred over the past several thousands of years (Doney and Schimel, 2007). Indeed, over just the past century the cumulative release of anthropogenic greenhouse gases has resulted in a mean rise in global air temperatures of 0.6 °C (Smith et al., 2009). As oceans serve as sinks for atmospheric heat, sea-surface temperatures (SST) have similarly risen on average 0.4 °C (Levitus et al., 2009), and it is now predicted that sea temperatures will climb another 2–3 °C by the end of the century (Collins et al., 2013). In addition to rising temperatures, the absorption of atmospheric CO₂ has resulted in ocean acidification (OA). To date, since the onset of the industrial revolution, mean ocean pH has declined on average by about 0.1 pH units (Rhein et al., 2013). Assuming a 'business as usual' model of fossil-fuel combustion, the pH of the world's oceans is expected to decline another 0.3–0.4 pH units by 2100 (Hoegh-Guldberg et al., 2014).

Temperature is a key environmental factor that affects multiple aspects of marine invertebrate physiology and ecology (Byrne et al., 2011; Pörtner, 2008). In some instances increased temperature within the range of thermal tolerance can be beneficial, for example, promoting rates of larval or juvenile development and growth (Delorme and Sewell, 2013; Hardy et al., 2014; Li et al., 2011). Moreover, when

temperature increases within an organism's optimum thermal range it can lessen the negative impacts of changes in other environmental factors such as pH (e.g., Brennan et al., 2010; Byrne et al., 2011; McCulloch et al., 2012) or salinity (Delorme and Sewell, 2014). In contrast, marine invertebrates living at or near their upper thermal tolerance may experience sub-lethal stress caused by elevated respiratory demands or other physiological functions that may limit resources and energy available for growth and reproduction (Pörtner, 2008; Pörtner and Knust, 2007; Whiteley and Mackenzie, 2016).

Marine invertebrates that have dispersive larvae and/or are mobile may be capable of escaping warming seas by extending their geographic range to more physiologically tolerable thermal environments (Jones et al., 2009, 2010; Spencer, 2008). However, sessile marine invertebrates that lack dispersive larvae, and/or mobile invertebrates that face geographic barriers, have few, if any, options other than to rely on their genotypic plasticity and adaptive capacity to respond to the changing thermal regime. Moreover, some marine invertebrates are at particularly high risk because some geographic regions are more prone to future warming. For example, tropical and subtropical regions of the northern hemisphere are expected to experience some of the greatest amounts of ocean warming (Collins et al., 2013).

Despite echinoderms generally being considered eurythermal, they are still impacted by changes in temperature (Moore, 1966). These impacts occur across all life-history stages and can influence aspects of

* Corresponding author.

E-mail address: mcclinto@uab.edu (J.B. McClintock).

<https://doi.org/10.1016/j.jembe.2018.08.007>

Received 27 April 2018; Received in revised form 29 July 2018; Accepted 25 August 2018

Available online 06 September 2018

0022-0981/ © 2018 Elsevier B.V. All rights reserved.

development, physiology, behavior, and under severe temperature stress, survival (e.g., Byrne et al., 2013; Delorme and Sewell, 2013; Hardy et al., 2014; Sewell and Young, 1999). One potentially sub-lethal impact of temperature that has received little experimental attention is how skeletal mineralogy may be influenced by chronic exposure to elevated temperature. Echinoderms construct their skeletons out of ‘high magnesium calcite’ (defined as > 4% mole MgCO_3) and vary with skeletal component (McClintock et al., 2017). This mineral complex is even more soluble than aragonite, the more soluble of the two polymorphs of calcium carbonate, calcite and aragonite (Vinogradov and Odum, 1953; Weber, 1973). Moreover, the level of magnesium in calcite is a contributing factor both to the solubility and mechanical properties of calcite (Ma et al., 2009; Long et al., 2014; Wang et al., 1997). As solubility increases, so does susceptibility to dissolution with increasing OA (Morse et al., 2006). While skeletal magnesium content in marine invertebrates is to some degree linked to taxonomic history, Chave (1954) found that the magnesium content of echinoderm skeletons increases with decreasing latitude. Subsequent studies have lent support to this latitudinal hypothesis (Andersson et al., 2008; Smith et al., 2016; Duquette et al., 2018; McClintock et al., 2011). While there is evidence that echinoderms exert biological controls over the magnesium content of their skeletal components (e.g., Gorzelak et al., 2013; Iglukowska et al., 2017; Robach et al., 2006; Veis, 2011), the magnesium content in a given species may also vary with a variety of other factors including salinity, the Mg/Ca ratio of seawater, carbonate saturation state, and diet (Andersson et al., 2008; Asnaghi et al., 2014; Marubini et al., 2003; Ries, 2004).

Wolfe et al. (2013) postulated that as temperature affects overall rate of growth, it is likely to be the most important factor determining skeletal magnesium content in echinoderms, and particularly for species inhabiting intertidal and shallow subtidal environments. To date, a number of studies have manipulated temperature to evaluate its impact on magnesium content in echinoid skeletal components (e.g., Hermans et al., 2010; Byrne et al., 2014; Smith et al., 2016; Collard et al., 2016). These studies have demonstrated a variety of outcomes from no effect to significant elevations in skeletal magnesium with exposure to increased temperature.

Lytechinus variegatus is a common sea urchin that occurs in shallow subtidal habitats. Its biogeographic distribution ranges along the Atlantic coast from Brazil to North Carolina, as well as throughout the Gulf of Mexico and Caribbean (Mortensen, 1943; Watts et al., 2001). Dense populations of *L. variegatus* inhabit seagrass beds where they are primary grazers, impacting seagrass shoot density and nutrient and energy transfer within seagrass communities (Heck and Valentine, 1995; Watts et al., 2013). Large populations occur in semi-enclosed bays in the Gulf of Mexico at the northern limit of their biogeographic range within the Gulf. Summer seawater temperature in these shallow bays averages about 26 °C (Beddingfield and McClintock, 2000). While populations of *L. variegatus* in the northern Gulf currently experience significant daily and seasonal variations in seawater temperature (Beddingfield and McClintock, 2000; Challener et al., 2015), individuals chronically exposed in the laboratory to temperatures of 30 °C or above experience signs of physiological stress, an indication that these populations are living near their upper thermal limit. For example, chronic exposure to 30.5 °C for 6–8 days reduces growth rate and survival in the echinoplutei larvae of *L. variegatus* from the Caribbean coast of Panama where average field temperatures are several degrees higher than the Gulf of Mexico (Collin and Chan, 2016). Adults collected in the Gulf of Mexico and exposed to 30 °C for 90 days reduce their feeding, allocate less carbohydrate to the Aristotle's lantern, and produce thinner spines (Brothers and McClintock, 2018). Behavior may also be impacted as righting and covering in *L. variegatus* are negatively impacted after a 10-day chronic exposure to 32 °C (Brothers and McClintock, 2015).

Informed by the findings of studies cited above the primary objective of the present study was to test the hypothesis that chronic elevated

near-future temperature impacts the skeletal mineralogy (Mg/Ca ratios) during the rapid juvenile growth phase of individuals of the common sea urchin *Lytechinus variegatus*. By focusing on juveniles we not only took advantage of their rapid growth but generally avoided the potential confounding effects of gamete production. Our a priori expectation was that if temperature did affect magnesium content, it would induce higher, rather than lower levels in skeletal components [as observed by Hermans et al., 2010 for *Paracentrotus lividus* up to a given plateau]. Importantly, our study expands the evaluation of the capacity of this species to withstand rapidly rising seawater temperatures in the northern Gulf of Mexico.

2. Methods

2.1. Field collections, initial analyses, and maintenance

Juveniles of *Lytechinus variegatus* (20–25 mm diameter, $n = 85$) were hand collected from shallow (1–2 m depth) seagrass beds in Eagle Harbor in Port Saint Joseph Bay, FL (29°45'N, 85°24'W) in June 2014 (adult size is considered to be approx. 35 mm diameter, Beddingfield and McClintock, 2000). Individuals were placed in coolers filled half-way with ambient seawater (27.7 °C as measured with an YSI 85 temperature meter) equipped with portable air bubblers and transported immediately to the University of Alabama at Birmingham. Upon return to the laboratory, all 85 sea urchins were numbered, and 25 individuals were randomly selected using a random number generator, measured (diameter, D), weighed (wet weight, g), and sacrificed for mineralogical analysis (see details below) to establish Time 0 (baseline) Mg/Ca ratios. The remaining sixty urchins were renumbered and, using a random number generator, randomly distributed equally into twenty 38-L aquaria (3 urchins per aquarium) containing artificial seawater (Instant Ocean® prepared with reverse-osmosis filtered water) at a salinity of 32 ppt and held at 26 °C and pH 8.2. Each urchin was placed in an individual plastic cylindrical cage (25.4 cm H × 10.2 cm D). Aquaria were aerated continuously with bubblers plumbed to CORALIFE® Super LuftSL-65 high pressure aquarium pumps, and seawater in each aquarium was circulated using a recirculating filter pump (Aqueon Power Filter 10). Water temperatures in each aquarium were controlled with EHEIM Jager 50-W heaters, and temperature monitored daily with a YSI 85 temperature meter. Sea urchin feces and uneaten artificial food (see below) were siphoned out of each cylindrical cage every day prior to feeding, and a 30–50% water exchange was carried out every other day to maintain good water quality. Physical parameters and water chemistry were monitored every other day using a Fisher Scientific handheld refractometer (salinity), Aquarium Pharmaceuticals, Inc. liquid test kit (ammonia, nitrate, nitrite), and a Fisher Scientific Accumet AB 15 pH meter equipped with an ACCUTU electrode (pH).

2.2. Experimental temperature treatments

Prior to initiating the two controlled temperature treatments, all sea urchins ($n = 60$) were held at 26 °C for one week to allow individuals to adjust to laboratory conditions following collection and transport. Each of the twenty aquaria were then randomly assigned to one of two temperature treatments: a mean summer seawater temperature of 26 °C for Saint Joseph Bay, Florida (Beddingfield and McClintock, 2000) or a predicted near-future elevated seawater temperature of 30 °C (Collins et al., 2013). This temperature was below the lethal limit for *L. variegatus* which is approximately 32 °C, (Brothers and McClintock, 2015). Sea urchins were fed an ad libitum diet of formulated sea urchin feed (Hammer et al., 2012) and the temperature in each near-future elevated seawater temperature aquarium was gradually increased over a one-week period to reach its target experimental temperature. Sea urchins were subsequently held at their respective temperature for a 90-day period under 12hL:12hD photoperiod. Every 30 days, each aquarium was cleaned to remove bacterial growth and algal biofilms (Challener

and McClintock, 2013).

2.3. Growth and skeletal magnesium calcite measurements

On day 30, 60, and 90 a sea urchin was haphazardly removed from each aquarium ($n = 10$ urchins per temperature treatment). Test diameter (D) was measured using calipers and whole body wet weight (wet wt) determined using a top-loading balance following a 30-s draining period on an absorbent paper towel. Each individual was then sacrificed using dissection scissors to cut the test horizontally into two halves. Tissues were cleaned from the test with forceps and the spines, test, and Aristotle's lantern isolated. All of the skeletal components were submerged in a 10% NaClO solution for at least 24 h to dissolve away remaining tissue. When necessary, the weak NaClO solution was exchanged to complete the digest. The cleaned skeletal components were rinsed with distilled water, vacuum dried, and air dried for 24 h. The entire dried skeletal components were ground into a fine powder using an agate mortar and pestle while adding approximately 15 drops of 95% ethanol to facilitate grinding. The resultant slurry was air dried for 24 h and approximately 30 mg of the dry powder was used in X-ray Diffraction Analysis (XRD).

XRD analyses were carried out using a Philips X'Pert Analytical X-ray diffraction system. The x-ray tube had a copper target and the wavelength utilized in the experiment was 1.54056 \AA (0.154056 nm). The resulting XRD pattern was used to determine the Mg/Ca ratio following the equation in Ries (2011):

$$\text{Mg/Ca} = 0.17881(2\theta)^2 - 10.20926(2\theta) + 145.59368.$$

The above equation relates Mg/Ca ratio to the 2θ value of the main calcite peak taken from the resulting XRD pattern.

2.4. Statistical analyses

Mg/Ca ratios were $\log(x + 1)$ transformed to fit a normal distribution. Mg/Ca ratios, test diameter, and wet weight were statistically compared over time within, as well as between, temperature treatments. Statistical comparisons over time did not include the Time Zero (baseline) data as it was collected prior to exposure to experimental temperatures. Mg/Ca ratios were also compared within temperature treatments among body components (spines, test, and lantern). A one-way repeated measures analysis of variance (RMANOVA) was used to determine if test D, whole body wet wt, or skeletal Mg/Ca ratio for body components changed with time. Sphericity was not assumed and the Geisser-Greenhouse correction was used in the RMANOVA. A Tukey HSD post-hoc test was employed to specify which time points differed from others. At each time point (30, 60, or 90 days), a Student's t -test was used to compare mean test D, whole body wet wt, or Mg/Ca ratios between the two temperature treatments. Lastly, a one-way analysis of variance (ANOVA) was used to determine if skeletal Mg/Ca ratios in the test, spines, and lantern were significantly different from one another at each time period sampled within each of the two temperature treatments. A Tukey HSD post-hoc test differentiated which body components were significantly different from the others.

3. Results

3.1. Growth

Four of the original 30 sea urchins in the near-future 30°C temperature treatment were removed from aquaria over the course of the experiment due to spine loss. The balance of 26 sea urchins in the 30°C temperature treatment appeared healthy (no spine loss, normal feeding). All sea urchins held in the ambient 26°C temperature treatment appeared healthy. Time (day 30, 60, and 90) had a significant effect in the 26°C and 30°C treatments on both the mean wet wt (RMANOVA; Geisser-Greenhouse's epsilon = 0.828, $F_{(1.66,14.9)} = 30.6$,

Table 1

Mean (\pm SE) total wet weight and test diameter of *Lytechinus variegatus* held for 90 days at either 26°C or 30°C and sub-sampled every 30 days. Sample sizes were 25 individuals at Time Zero (baseline), 10 individuals at each time point in the 26°C treatment, and 10 individuals at day 30 and 8 individuals at days 60 and 90 in the 30°C treatment.

Time (days)	Wet weight (g)		Test diameter (mm)	
	26°C	30°C	26°C	30°C
Baseline	7.02 ± 0.45		24.04 ± 0.56	
30	17.89 ± 0.77	17.58 ± 0.83	32.0 ± 0.8	31.3 ± 0.5
60	24.56 ± 1.26	23.16 ± 0.72	36.9 ± 0.4	36.5 ± 0.3
90	31.87 ± 1.59	28.14 ± 0.78	39.7 ± 0.9	37.9 ± 0.5

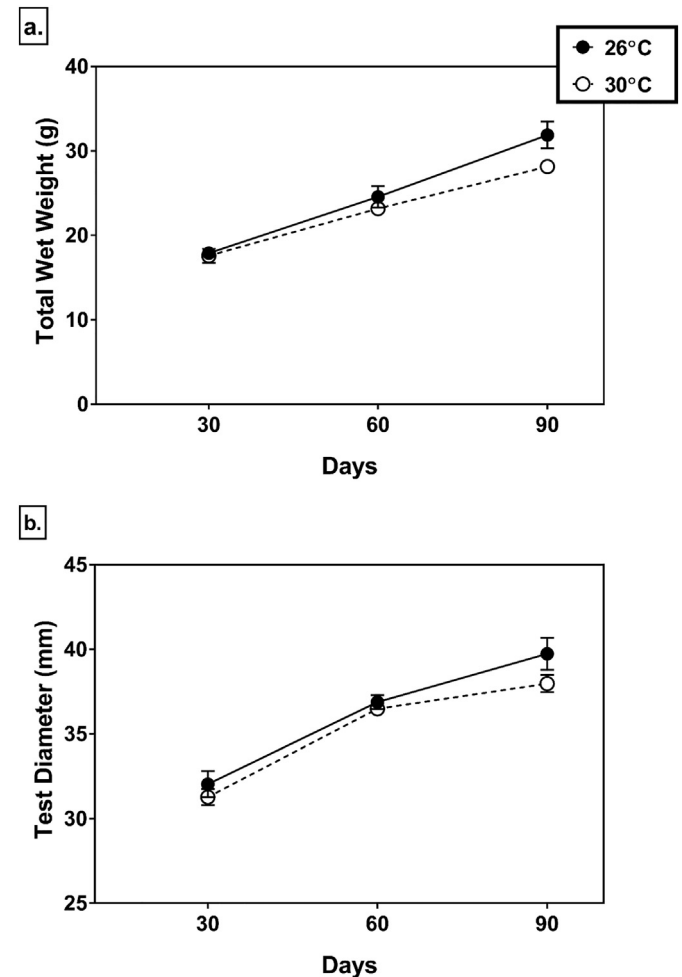


Fig. 1. Growth of individuals of *Lytechinus variegatus* held at either 26°C (filled circles) or 30°C (open circles) for 90 days and fed an ad libitum diet of formulated sea urchin food. Measurements were taken at 30, 60, and 90 days. Growth is presented by change in both wet wt (a) and test diameter (b). Error bars show means \pm 1 SE. Time Zero ($n = 25$), 26°C ($n = 10$), 30°C ($n = 10$ at day 30, $n = 8$ at days 60 and 90).

$p < 0.001$; Geisser-Greenhouse's epsilon = 0.936, $F_{(1.87,13.1)} = 53.3$, $p < 0.001$, respectively) and the mean test D of the sea urchins (RMANOVA; Geisser-Greenhouse's epsilon = 0.846, $F_{(1.69,15.2)} = 24.3$, $p < 0.001$; Geisser-Greenhouse's epsilon = 0.895, $F_{(1.79,12.5)} = 62.7$, $p < 0.001$, respectively). Both mean wet wt and mean test D increased with time (Table 1; Fig. 1a,b). A Tukey HSD test indicated that, within the 26°C treatment, mean wet wt differed significantly between each time point (Tukey HSD test; 30 vs. 60 $p = 0.003$; 30 vs. 90 $p < 0.001$; 60 vs 90 $p = 0.019$). Similarly, within the 30°C treatment, mean wet wt

of sea urchins was significantly different at every time point (Tukey HSD test; 30 vs. 60 $p = 0.003$; 30 vs. 90 $p < 0.001$; 60 vs 90 $p = 0.002$), but the increase between days 30 and 60 (5.6 g mean wet wt) was greater than the difference between days 60 and 90 (5.0 g mean wet wt). Sea urchins held in the 26 °C seawater demonstrated significant mean test diameter increase at each time point (Tukey HSD test; $p < 0.05$). However, sea urchins held in the 30 °C seawater demonstrated a significant mean test D differences between the 30 and 60 day measurement and the 30 and 90 day measurement (Tukey HSD test; $p < 0.001$). In contrast, mean test D from day 60 to 90 were not significantly different from one another (Tukey HSD test; $p = 0.052$). Elevated seawater temperature (30 °C) did not have a significant effect on either mean body wet wt (t -test, day 30: $t_{(18)} = 0.112$, $p = 0.912$; day 60: $t_{(16)} = 0.897$, $p = 0.382$; day 90: $t_{(16)} = 1.944$, $p = 0.070$) or mean test D in sea urchins (t -test; day 30: $t_{(18)} = 0.133$, $p = 0.896$; day 60: $t_{(16)} = 0.720$, $p = 0.482$; day 90: $t_{(16)} = 1.515$, $p = 0.149$) when compared to those in the ambient temperature treatment.

3.2. Skeletal component Mg/Ca ratios

Intra-skeletal component Mg/Ca ratios did not vary over time within either the 26 °C or the 30 °C temperature treatment for the spines (RMANOVA; Geisser-Greenhouse's epsilon = 0.868, $F_{(1.74,15.6)} = 0.676$, $p = 0.503$; Geisser-Greenhouse's epsilon = 0.735, $F_{(1.47,10.3)} = 1.67$, $p = 0.232$, respectively; Fig. 2a), test (RMANOVA; Geisser-Greenhouse's epsilon = 0.987, $F_{(1.97,17.8)} = 0.411$, $p = 0.666$; Geisser-Greenhouse's epsilon = 0.571, $F_{(1.17,7.99)} = 0.96$, $p = 0.370$, respectively, Fig. 2b), or lantern (RMANOVA; Geisser-Greenhouse's epsilon = 0.653, $F_{(1.31,11.8)} = 0.0295$, $p = 0.658$; Geisser-Greenhouse's epsilon = 0.958, $F_{(1.92,13.4)} = 0.365$, $p = 0.692$, respectively; Fig. 2c).

Sea urchins in the 26 °C treatment had consistently higher mean Mg/Ca ratios than sea urchins held at 30 °C. The Mg/Ca ratios differed for each body component (Table 2). For the spines, mean Mg/Ca ratios differed significantly between treatments on day 30 (Student t -test; $t_{(18)} = 2.55$, $p = 0.020$; Fig. 2a) but were not significantly different on days 60 and 90. For the test, the significant difference in mean Mg/Ca ratios between the two temperature treatments occurred on day 60 (Student t -test; $t_{(16)} = 3.61$, $p = 0.002$; Fig. 2b). In contrast to the spines and test, the Aristotle's lantern demonstrated significant differences in mean Mg/Ca ratios between temperature treatments on days 30, 60, and 90 (Student t -test; $t_{(18)} = 3.94$, $p = 0.001$; $t_{(16)} = 2.553$, $p = 0.021$; $t_{(16)} = 2.337$, $p = 0.033$, respectively; Fig. 2c).

Mean Mg/Ca ratios were also compared among the body components within each temperature treatment (26 °C and 30 °C) and at each time point (30, 60, and 90 days, Fig. 3a,b,c). In the ambient 26 °C treatment, the spines had significantly lower mean ratios than did the test or lantern on days 30, 60, and 90 (ANOVA: $F_{(2,27)} = 15.68$, $p < 0.001$; $F_{(2,27)} = 9.19$, $p = 0.001$; $F_{(2,27)} = 14.02$, $p < 0.001$, respectively; Fig. 3a). In the elevated 30 °C treatment, the spines had significantly lower mean ratios than the test and lantern, but not at every time point (Fig. 3b,c). The skeletal material in the three body components of individuals held at 30 °C did not have significantly different mean Mg/Ca ratios at day 60 (ANOVA: $F_{(2,21)} = 1.02$, $p = 0.378$), but did at days 30 and 90 (ANOVA: $F_{(2,21)} = 15.59$, $p < 0.001$; $F_{(2,21)} = 7.79$, $p = 0.003$, respectively).

4. Discussion

Individuals of juvenile *Lytechinus variegatus* examined in the present study were collected from similar habitats and maintained under controlled laboratory conditions that were identical in every respect except for seawater temperature. Accordingly, any differences we observed in growth and skeletal Mg/Ca ratios between treatments should be attributable to temperature. With respect to skeletal Mg/Ca ratios, we are aware that our approach did not distinguish between new and old skeletal growth, but we feel our results (see below) are convincing as

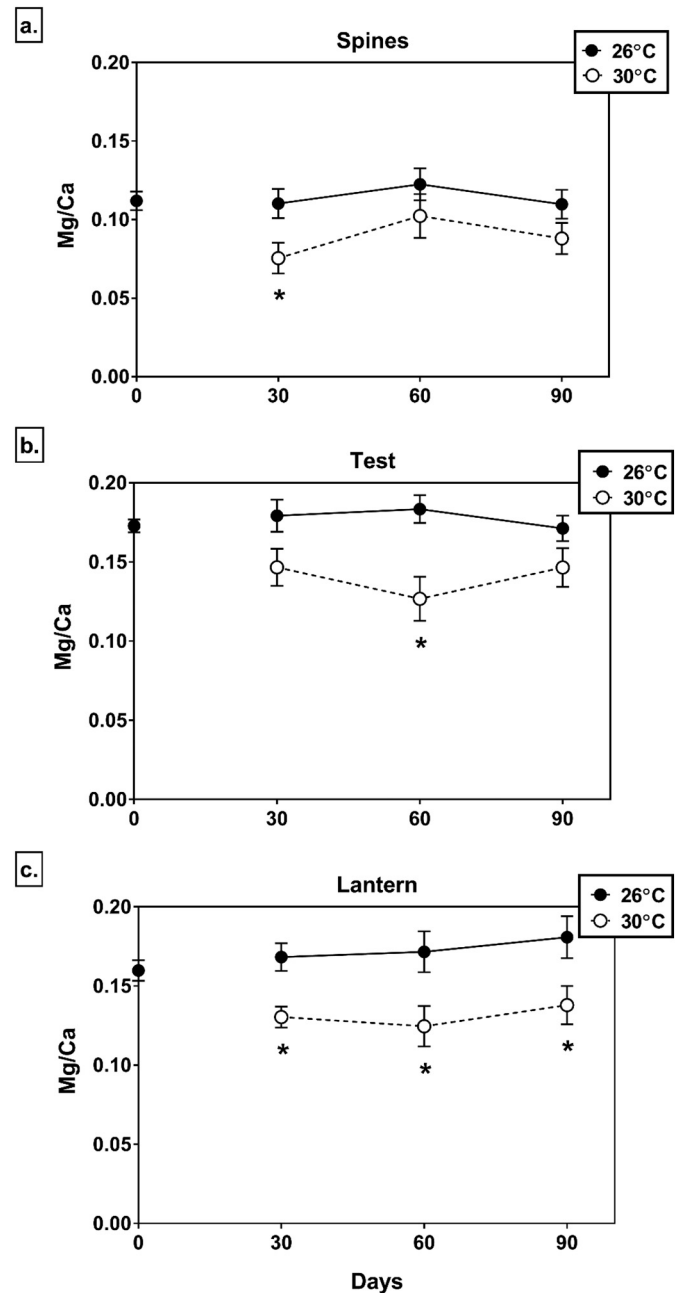


Fig. 2. Mg/Ca ratios for skeletal components (spines, test, and Aristotle's lantern) of *Lytechinus variegatus* held at either 26 °C (filled circles) or 30 °C (open circles) for 90 days and sub-sampled on days 30, 60, and 90. Data are presented for spines (a), test (b) and Aristotle's lantern (c). Error bars show means \pm 1 SE. Asterisks denote statistically significant differences between temperature treatments. Time Zero ($n = 25$), 26 °C ($n = 10$), 30 °C ($n = 10$ at day 30, $n = 8$ at days 60 and 90).

the biogeochemical signatures of new growth in skeletal parts appears to have overridden those in older growth. Rapid increases in body mass and test diameter in *L. variegatus* in both temperature treatments were similar to those in the field (Beddingfield and McClintock, 2000) indicating sea urchins were in good condition and actively growing. There were small but non-significant declines in mean body wet weight and test diameter between days 60 and 90 at 30 °C. It is possible this is because chronic exposure to elevated temperature had begun to be sufficiently stressful to begin to hinder growth. This may also explain why individuals in the elevated temperature treatment did not grow faster and attain a larger body size than those held at the ambient

Table 2

Mean (\pm SE) molar Mg/Ca ratios of *Lytechinus variegatus* held at either 26 °C or 30 °C for 90 days and sub-sampled every 30 days. Sample size was 25 individuals at Time Zero (baseline), 10 individuals at each time point in the 26 °C treatment, and 10 individuals at day 30 and 8 individuals at days 60 and 90 in the 30 °C treatment.

Body component	Day	Mean molar Mg/Ca (\pm SE)	
		26 °C	30 °C
Spines	Baseline	0.046 \pm 0.005	
	30	0.110 \pm 0.009	0.075 \pm 0.010
	60	0.122 \pm 0.010	0.102 \pm 0.014
	90	0.110 \pm 0.009	0.088 \pm 0.010
Test	Baseline	0.066 \pm 0.002	
	30	0.179 \pm 0.010	0.147 \pm 0.012
	60	0.183 \pm 0.009	0.127 \pm 0.014
	90	0.171 \pm 0.008	0.147 \pm 0.012
Lantern	Baseline	0.063 \pm 0.004	
	30	0.168 \pm 0.009	0.130 \pm 0.007
	60	0.172 \pm 0.013	0.125 \pm 0.013
	90	0.181 \pm 0.013	0.138 \pm 0.012

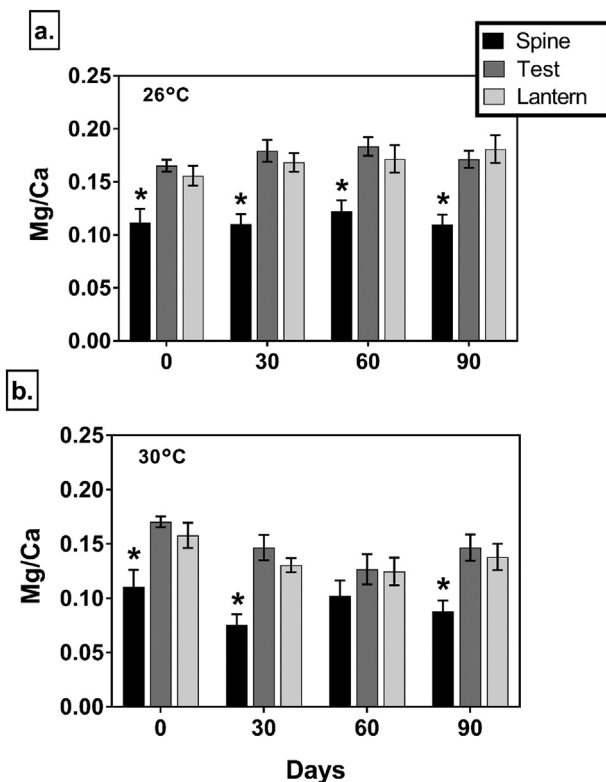


Fig. 3. Mg/Ca ratios for major skeletal components (spines, test, Aristotle's lantern) of *Lytechinus variegatus* held at either 26 °C (a) or 30 °C (b) for 90 days and sub-sampled on days, 30, 60, and 90. The black bar represents spines, the gray bar represents the test, and the lightest bars represent the Aristotle's lantern. Error bars show means \pm 1 SE. Asterisks indicate statistically significant differences between skeletal components. Time Zero (n = 25), 26 °C (n = 10), 30 °C (n = 10 at day 30, n = 8 at days 60 and 90).

temperature, despite unlimited food availability. Longer term studies are needed to further examine this possibility. Temperature has well-established impacts on echinoderm physiology including the stimulation of growth within an optimal thermal range (Fujisawa and Shigei, 1990; Hardy et al., 2014). However, if temperature exceeds an individual's thermal tolerance, benefits of an increased temperature such as an elevated metabolic rate are constrained. Reduced metabolism limits energy available for physiological processes beyond

maintenance, including growth and reproduction (Pörtner, 2008).

Unlike observed changes in mass and test diameter, we observed no significant intra-skeletal changes in mean Mg/Ca ratios within the spines, test, or Aristotle's lantern in individuals held in either temperature treatment over the course of the experiment. Accordingly, at least for *Lytechinus variegatus*, there appear to be no post-metamorphic ontogenetic changes in mineralogy (Mg/Ca ratios) within a given skeletal component. Individuals in ambient and elevated temperature grew from juvenile (approx. 10 g wet wt) to adult (approx. 29 g wet wt) body size, a threefold increase in body mass over the 90-day experiment. This rate of growth is rapid compared with other species of regular sea urchins (Lawrence, 2013) and similar to that of juvenile *Lytechinus variegatus* in the field under optimal conditions indicating that individuals in the present study were not stressed (Beddingfield and McClintock, 2000).

Overall, temperature had a clear and significant impact on levels of calcitic magnesium in the sea urchin *Lytechinus variegatus*. By day 30 of the experiment, individuals chronically exposed to 30 °C had mean Mg/Ca ratios in all three major skeletal components that were significantly lower (18–35%) than those measured in individuals held at 26 °C for the same time period. Mean Mg/Ca ratios measured in skeletal components of sea urchins at Time Zero (time of field collection) indicated that at the time of collection, Mg/Ca ratios were similar to those of urchins held in the ambient 26 °C treatment over the entire course of the experiment. On day 60, ratios of Mg/Ca were again lower in all three skeletal components for individuals held in the elevated temperature treatment, trending lower in the spines, but significantly lower in both the test and lantern. By Day 90 there was still a consistent pattern of lower ratios of Mg/Ca in all skeletal components in the elevated temperature, with Mg/Ca ratios for the test and spines trending lower, and the lantern significantly lower than skeletal components of urchins held in the ambient temperature treatment. Our findings contrast with those of Hermans et al. (2010) who found that Mg/Ca ratios in the skeleton of the sea urchin *Paracentrotus lividus* increased with increasing temperature up until a plateau at a high, but field occurring, temperature. They postulated that this plateau is caused by properties of the organic matrix of mineralization. This hypothesis provides a potential explanation for the lack of an increase in Mg/Ca ratios we observed in skeletal elements of *L. variegatus* chronically exposed to near-future elevated temperature. We are unaware, however, of a mechanistic explanation to postulate for the reduction in Mg/Ca ratios we observed.

Weber (1973) conducted analyses of magnesium in the skeletal calcite of a large sample of species of echinoids and asteroids to re-examine the long presumed geological significance of the relationship between magnesium content and water temperature. He concluded that variations in skeletal chemistry suggested that physiological factors, possibly related to calcification rate, were more important than environmental factors in controlling magnesium levels in the calcium carbonate deposited by echinoderms. If calcification rate is indeed a regulatory factor in the processes driving calcitic magnesium levels, our study suggests that stress in response to exposure to chronic elevated temperature impeded this rate-dependent process. As urchins in both our temperature treatments had no significant differences in growth rate, somatic growth rate can be eliminated as having influenced the differences we detected in skeletal Mg/Ca ratios between the two temperature treatments. Regardless of whether magnesium concentration is reduced in individuals chronically exposed to elevated temperature because to an indirect physiological response, or a direct response related to mineral formation, the functional consequences remain the same for *L. variegatus* coping with near-future warming.

Our findings are similar to previous studies that report discrete skeletal components of sea urchins (e.g., test, spines, and lantern) possess concentrations of magnesium that differ significantly from one another (Smith et al., 2016; Iglikowska et al., 2017, 2018). Typically, echinoid spines contain the lowest levels of magnesium (Borzęcka-

Prokop et al., 2007; McClintock et al., 2011, 2017), a pattern that was confirmed in the present study for *Lytechinus variegatus*, whose spines had Mg/Ca ratios approximately 35% lower than either the test or lantern. Sea urchin skeletal components have evolved for different functions and biomineralization processes have accommodated the capacity to alter mineralogy to address these selective pressures (Goetz et al., 2014; Robach et al., 2009; Veis, 2011). It has been shown that in sea urchin teeth in particular, mineral hardness can be increased with increasing magnesium content (Ma et al., 2009; Wang et al., 1997). Elevated hardness of the lantern may reduce erosion rates and enhance the self-sharpening nature of the teeth within the lantern (Robach et al., 2009; Wang et al., 1997). It is possible that the higher magnesium content of the test contributes to its strength as well. Increased hardness of the interlocking tabular ossicles or plates that comprise the test may improve resistance against crushing predators. However, test strength does also depend on the soft collagenous tissue ligaments that bind together the numerous plates make up the test (Ellers et al., 1998). At first glance it is puzzling why the protective spines of *L. variegatus* and many other echinoids are not under similar selective pressure as the test and lantern for higher levels of magnesium. This may be explained by echinoid spines having a unique microstructure of single-crystal calcite that is organized in an intricate morphology that provides both lightness and hardness (Tsafnat et al., 2012).

Despite the significant inverse correlation between skeletal magnesium content and latitude long documented in echinoderms (Chave, 1954), the potential abiotic or biotic factors driving this relationship remain speculative (McClintock et al., 2011; Duquette et al., 2018). Our manipulation of temperature in the present study provides an opportunity to consider this environmental factor as a candidate for its potential role in regulating skeletal magnesium content (Chave, 1954; Duquette et al., 2018; Wolfe et al., 2013). Indeed, temperature seems a likely factor as latitude is a strong proxy for sea-surface temperature (Smith et al., 2016). However, while we found that chronic exposure to elevated temperature resulted in a significant reduction on calcitic magnesium in *Lytechinus variegatus*, warmer-water echinoderms have a higher skeletal magnesium content than do colder-water species (Chave, 1954). Our findings do not, therefore, provide unequivocal evidence for a potential role for temperature in driving the intriguing relationship between latitude and skeletal magnesium content in echinoderms.

There are important functional implications as well as cost-benefit trade-offs related to chronic exposure to elevated temperature causing significant reductions in skeletal magnesium content in *Lytechinus variegatus*. Although not examined in the present study, reduced levels of calcitic magnesium may contribute to lower skeletal hardness, an outcome that is particularly relevant for the test because it provides protection against skeletal crushing predators including crabs and some fish. On the other hand, decreased levels of skeletal magnesium reduces susceptibility to dissolution under intensifying ocean acidification. Accordingly, up to some thermal maxima, seawater temperatures could help offset near-future impacts of ocean acidification in *L. variegatus*. This is important, as echinoderms experiencing OA-induced skeletal dissolution are likely to invest their limited resources in skeletal maintenance and, by doing so, compromise fitness (Wood et al., 2010).

To better understand and predict how *L. variegatus* will be affected by and respond to climate changes, future studies should endeavor to use multifactorial experimental designs investigating both temperature and acidification effects. While this study demonstrated that ocean warming may lead to a decrease in magnesium content of echinoderm skeletal components, especially in organisms already living near the top of their thermal tolerance like *L. variegatus*, it is unknown if that decrease would be altered by exposure to acidification as well. Ocean warming and acidification will occur in conjunction with one another and, therefore, studies testing both stressors offer more predictive power as to how organisms will respond to on-going climate changes.

Acknowledgements

The authors thank Stephen Watts and Jeff Barry for providing formulated sea urchin feed. They also thank Gopi Samudrala for technical assistance with the XRD analyses. Support for this study was provided by the UAB Department of Biology and an Endowed Professorship in Polar and Marine Biology provided to JBM.

References

- Andersson, A.J., Andersson, A.J., Mackenzie, F.T., Bates, N.R., 2008. Life on the margin: implications of ocean acidification on Mg-calcite, high latitude and cold-water marine calcifiers. *Mar. Ecol. Prog. Ser.* 373, 265–273.
- Asnaghi, V., Mangialajo, L., Gattuso, J.P., Francour, P., Privitera, D., Chiantore, M., 2014. Effects of ocean acidification and diet on thickness of carbonate elemental composition of the test of juvenile sea urchins. *Mar. Environ. Res.* 93, 78–84.
- Beddingfield, S.D., McClintock, J.B., 2000. Demographic characteristics of *Lytechinus variegatus* (Echinoidea: Echinodermata) from three habitats in a North Florida Bay, Gulf of Mexico. *Mar. Ecol. Prog. Ser.* 21, 17–40.
- Borzecka-Prokop, B., Weselucha-Birczyńska, A., Koszowska, E., 2007. MicroRaman, PXRD, EDS and microscopic investigation of magnesium calcite biomineral phases. The case of sea urchin biominerals. *J. Mol. Struct.* 828, 80–90.
- Brennand, H.S., Soars, N., Dworjanyn, S.A., Davis, A.R., Byrne, M., 2010. Impact of ocean warming and ocean acidification on larval development and calcification in the sea urchin *Tripeustes gratilla*. *PLoS One* 5, e11372.
- Brothers, C., McClintock, J., 2015. The effects of climate-induced elevated seawater temperature on the covering behavior, righting response, and Aristotle's lantern reflex of the sea urchin *Lytechinus variegatus*. *J. Exp. Mar. Biol. Ecol.* 467, 33–38.
- Brothers, C.J., McClintock, J.B., 2018. Sea urchins exposed to near-future elevated seawater temperature alter resource allocation under low-quality food conditions. *Mar. Biol.* 165, 43.
- Byrne, M., Ho, M., Wong, E., Soars, N.A., Selvakumaraswamy, P., Shepard Brennand, H., Dworjanyn, S.A., Davis, A.R., 2011. Unshelled abalone and corrupted urchins: development of marine calcifiers in a changing ocean. *Proceedings of the Royal Soc. Lond. B: Biol. Sci.* 278, 2376–2383.
- Byrne, M., Foo, S., Soars, N.A., Wolfe, K.D., Nguyen, H.D., Hardy, N., Dworjanyn, S.A., 2013. Ocean warming will mitigate the effects of acidification on calcifying sea urchin larvae (*Heliocidaris tuberculata*) from the Australian global warming hot spot. *J. Exp. Mar. Biol. Ecol.* 448, 250–257.
- Byrne, M., Smith, A.M., West, S., Collard, M., Dubois, P., Graba-landry, A., Dworjanyn, S.A., 2014. Warming influences Mg²⁺ content, while warming and acidification influence calcification and test strength of a sea urchin. *Environ. Sci. Technol.* 48 (12), 620–627.
- Challener, R.C., McClintock, J.B., 2013. Exposure to extreme hypercapnia under laboratory conditions does not impact righting and covering behavior of juveniles of the common sea urchin *Lytechinus variegatus*. *Mar. Freshw. Behav. Physiol.* 46, 191–199.
- Challener, R.C., Robbins, L.L., McClintock, J.B., 2015. Variability of the carbonate chemistry in a shallow, seagrass-dominated ecosystem: implications for ocean acidification experiments. *Mar. Freshw. Res.* 67, 163–172.
- Chave, K.E., 1954. Aspects of the biogeochemistry of magnesium 1. Calcareous marine organisms. *J. Geol.* 62, 266–283.
- Collard, M., Rastrick, S.P., Calosi, P., Demolder, Y., Dille, J., Findlay, H.S., Hall-Spencer, J.M., Milazzo, M., Moulin, L., Widdicombe, S., Dehairs, F., 2016. The impact of ocean acidification and warming on the skeletal mechanical properties of the sea urchin *Paracentrotus lividus* from laboratory and field observations. *ICES J. Mar. Sci.* 73, 727–738.
- Collin, R., Chan, K.Y.K., 2016. The sea urchin *Lytechinus variegatus* lives close to the upper thermal limit for early development in a tropical lagoon. *Ecol. Evol.* 6, 5623–5634.
- Collins, M., Knutti, R., Arblaster, J., Dufresne, J.-L., Fichet, T., Friedlingstein, P., Gao, X., Gutowski, W.J., Johns, T., Krinner, G., Shongwe, M., Tebaldi, C., Weaver, A.J., Wehner, M., 2013. Long-term Climate Change: Projections, Commitments and Irreversibility. In: Stocker, T.F., Qin, D., Plattner, G.-K., Tignor, M., Allen, S.K., Boschung, J., Nauels, A., Xia, Y., Bex, V., Midgley, P.M. (Eds.), *Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, pp. 1029–1136.
- Delorme, N.J., Sewell, M.A., 2013. Temperature limits to early development of the New Zealand sea urchin *Evechinus chloroticus* (Valenciennes, 1846). *J. Therm. Biol.* 38, 218–224.
- Delorme, N.J., Sewell, M.A., 2014. Temperature and salinity: two climate change stressors affecting early development of the New Zealand sea urchin *Evechinus chloroticus*. *Mar. Biol.* 161, 1999–2009.
- Doney, S.C., Schimel, D.S., 2007. Carbon and climate system coupling on timescales from the precambrian to the anthropocene. *Annu. Rev. Environ. Resour.* 32, 31–66.
- Duquette, A., Halanach, K., Angus, R., McClintock, J., 2018. Inter and intraspecific comparisons of the skeletal Mg/Ca ratios of high latitude Antarctic echinoderms. *Antarct. Sci.* 30, 160–169.
- Ellers, O., Johnson, A.S., Moberg, P.E., 1998. Structural strengthening of urchin skeletons by collagenous sutural ligaments. *Biol. Bull.* 195, 136–144.
- Fujisawa, H., Shigei, M., 1990. Correlation of embryonic temperature sensitivity of sea urchins with spawning season. *J. Exp. Mar. Biol. Ecol.* 136, 123–139.

- Goetz, A.J., Griesshaber, E., Abel, R., Fehr, T., Ruthensteiner, B., Schmahl, W., 2014. Tailored order: the mesocrystalline nature of sea urchin teeth. *Acta Biomater.* 10, 3885–3898.
- Gorzalack, P., Stolarski, J., Mazur, M., Meibom, A., 2013. Micro-to nanostructure and geochemistry of extant crinoidal echinoderm skeletons. *Geobiology* 11, 29–43.
- Hammer, H.S., Powell, M.L., Jones, W.T., Gibbs, V.K., Lawrence, A.L., Lawrence, J.M., Watts, S.A., 2012. Effect of feed protein and carbohydrate levels on feed intake, growth, and gonad production of the sea urchin *Lytechinus variegatus*. *J. World Aquaculture Soc.* 43, 145–158.
- Hardy, N.A., Lamare, M., Uthicke, S., Wolfe, K., Doo, S., Dworjanyn, S., Byrne, M., 2014. Thermal tolerance of early development in tropical and temperate sea urchins: inferences for the tropicalization of eastern Australia. *Mar. Biol.* 161, 395–409.
- Heck, K.L., Valentine, J.F., 1995. Sea urchin herbivory: evidence for long-lasting effects in subtropical seagrass meadows. *J. Exp. Mar. Biol. Ecol.* 189, 205–217.
- Hermans, J., Borremans, C., Willenz, P., André, L., Dubois, P., 2010. Temperature, salinity and growth rate dependences of Mg/Ca and Sr/Ca ratios of the skeleton of the sea urchin *Paracentrotus lividus* (Lamarck): an experimental approach. *Mar. Biol.* 157, 1293–1300.
- Hoegh-Guldberg, O., Cai, R., Poloczanska, E.S., Brewer, P.G., Sundby, S., Hilmi, K., Fabry, V.J., Jung, S., 2014. The ocean. In: Barros, V.R., Field, C.B., Dokken, D.J., Mastrandrea, M.D., Mach, K.J., Bilir, T.E., Chatterjee, M., Ebi, K.L., Estrada, Y.O., Genova, R.C., Girma, B., Kissel, E.S., Levy, A.N., MacCracken, S., Mastrandrea, P.R., White, L.L. (Eds.), *Climate Change 2014: Impacts, Adaptation, and Vulnerability. Part B: Regional Aspects. Contribution of Working Group II to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*, Cambridge, United Kingdom and New York, NY, USA, pp. 1655–1731.
- Iglukowska, A., Najorka, J., Voronkov, A., Chelchowski, M., Kukliński, P., 2017. Variability in magnesium content in Arctic echinoderm skeletons. *Mar. Environ. Res.* 129, 207–218.
- Iglukowska, A., Borszcz, T., Drewnik, A., Grabowska, M., Humphreys-Williams, E., Kędra, M., Krzemińska, M., Piwoni-Piórewicz, A., Kukliński, P., 2018. Mg and Sr in Arctic echinoderm calcite: nature or nurture? *J. Mar. Syst.* 180, 279–288.
- Jones, S.J., Mieszkowska, N., Wetthey, D.S., 2009. Linking thermal tolerances and biogeography: *Mytilus edulis* (L.) at its southern limit on the east coast of the United States. *Biol. Bull.* 217, 73–85.
- Jones, S.J., Lima, F.P., Wetthey, D.S., 2010. Rising environmental temperatures and biogeography: poleward range contraction of the blue mussel, *Mytilus edulis* L., in the western Atlantic. *J. Biogeogr.* 37, 2243–2259.
- Lawrence, J.M., 2013. Sea urchins: biology and ecology. In: *Developments in Aquaculture and Fisheries Science*. vol. 38. Elsevier, pp. 531.
- Levitus, S., Antonov, J., Boyer, T., Locarnini, R., Garcia, H., Mishonov, A., 2009. Global ocean heat content 1955–2008 in light of recently revealed instrumentation problems. *Geophys. Res. Lett.* 36 (L07608).
- Li, L., Li, Q., Sun, X., Kong, L., 2011. Effects of temperature and salinity on larval growth, survival, and development of the sea cucumber *Apostichopus japonicus*. *N. Amer. J. Aquacult.* 73, 296–303.
- Long, X., Ma, Y., Qi, L., 2014. Biogenic and synthetic high magnesium calcite – a review. *J. Struct. Biol.* 185, 1–14.
- Ma, Y., Aichmayer, B., Paris, O., Fratzl, P., Meibom, A., Metzler, R.A., Politi, Y., Addadi, L., Gilbert, P., Weiner, S., 2009. The grinding tip of the sea urchin tooth exhibits exquisite control over calcite crystal orientation and Mg distribution. *Proc. Nat. Acad. Sci.* 106, 6048–6053.
- Marubini, F., Ferrier-Pages, C., Cuif, J.P., 2003. Suppression of skeletal growth in scleractinian corals by decreasing ambient carbonate-ion concentration: a cross-family comparison. *Proc. R. Soc. Lond. B* 270, 179–184.
- McClintock, J.B., Amsler, M.O., Angus, R.A., Challener, R.C., Schram, J.B., Amsler, C.D., Mah, C.L., Cuce, J., Baker, B.J., 2011. The Mg-calcite composition of Antarctic echinoderms: important implications for predicting the impacts of ocean acidification. *J. Geol.* 119, 457–466.
- McClintock, J., Amsler, M., Angus, R., McClintock, F., Geneviere, A.-M., Lebrato, M., 2017. A comparison of MgCO₃ in individual sub-components of major skeletal structures of the common temperate sea urchin *Paracentrotus lividus*. *Cah. Biol. Mar.* 58, 99–106.
- McCulloch, M., Falter, J., Trotter, J., Montagna, P., 2012. Coral resilience to ocean acidification and global warming through pH up-regulation. *Nat. Clim. Chang.* 2, 623–627.
- Moore, H.B., 1966. Ecology of echinoids. In: Boolootian, R.A. (Ed.), *Physiology of Echinodermata*. Interscience Publishers, New York.
- Morse, J.W., Andersson, A.J., Mackenzie, F.T., 2006. Initial responses of carbonate-rich shelf sediments to rising atmospheric pCO₂ and “ocean acidification”: role of high Mg-calcites. *Geochim. Cosmochim. Acta* 70, 5814–5830.
- Mortensen, T., 1943. A Monograph of the Echinoidea. II. 2. Camarodonta. I. CA Reitzel. (Copenhagen).
- Pörtner, H.-O., 2008. Ecosystem effects of ocean acidification in times of ocean warming: a physiologist's view. *Mar. Ecol. Prog. Ser.* 373, 203–217.
- Pörtner, H.O., Knust, R., 2007. Climate change affects marine fishes through the oxygen limitation of thermal tolerance. *Science* 315, 95–97.
- Rhein, M., Rintoul, S.R., Aoki, S., Campos, E., Chambers, D., Feely, R.A., Gulev, S., Johnson, G.C., Josey, S.A., Kostianoy, A., Mauritzen, C., Roemmich, D., Talley, L.D., Wang, F., 2013. Observations: ocean. In: Stocker, T.F., Qin, D., Plattner, G.-K., Tignor, M., Allen, S.K., Boschung, J., Nauels, A., Xia, Y., Bex, V., Midgley, P.M. (Eds.), *Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, pp. 255–316.
- Ries, J.B., 2004. Effect of ambient Mg/Ca ratio on Mg fractionation in calcareous marine invertebrates: A record of the oceanic Mg/Ca ratio over the Phanerozoic. *Geology* 32, 981–984.
- Ries, J.B., 2011. Skeletal mineralogy in a high-CO₂ world. *J. Exp. Mar. Biol. Ecol.* 403, 54–64.
- Robach, J., Stock, S., Veis, A., 2006. Mapping of magnesium and of different protein fragments in sea urchin teeth via secondary ion mass spectroscopy. *J. Struct. Biol.* 155, 87–95.
- Robach, J., Stock, S., Veis, A., 2009. Structure of first-and second-stage mineralized elements in teeth of the sea urchin *Lytechinus variegatus*. *J. Struct. Biol.* 168, 452–466.
- Sewell, M.A., Young, C.M., 1999. Temperature limits to fertilization and early development in the tropical sea urchin *Echinometra lucunter*. *J. Exp. Mar. Biol. Ecol.* 236, 291–305.
- Smith, K., Ruhl, H., Bett, B., Billett, D., Lampitt, R., Kaufmann, R., 2009. Climate, carbon cycling, and deep-ocean ecosystems. *Proc. Natl. Acad. Sci.* 106 (19), 211–218.
- Smith, A.M., Clark, D.E., Lamare, M.D., Winter, D.J., Byrne, M., 2016. Risk and resilience: variations in magnesium in echinoid skeletal calcite. *Mar. Ecol. Prog. Ser.* 561, 1–16.
- Spencer, P.D., 2008. Density-independent and density-dependent factors affecting temporal changes in spatial distributions of eastern Bering Sea flatfish. *Fish. Oceanogr.* 17, 396–410.
- Tsafnat, N., Gerald, J.D.F., Le, H.N., Stachurski, Z.H., 2012. Micromechanics of sea urchin spines. *PLoS One* 7, e44140.
- Veis, A., 2011. Organic matrix-related mineralization of sea urchin spicules, spines, test and teeth. *Front. Bioscience* 16, 2540–2560.
- Vinogradov, A.P., Odum, V., 1953. The elementary chemical composition of marine organisms, Memoir sears foundation for marine research No. 11. New Haven 677.
- Wang, R., Addadi, L., Weiner, S., 1997. Design strategies of sea urchin teeth: structure, composition and micromechanical relations to function. *Phil. Trans. Royal Soc. London. B: Biol. Sci.* 352, 469–480.
- Watts, S.A., McClintock, J.B., Lawrence, J.M., 2001. The ecology of *Lytechinus variegatus*. *Develop. Aqua. Fisheries Sci.* 32, 375–393.
- Watts, S., McClintock, J., Lawrence, J., 2013. *Lytechinus*. In: Lawrence, J. (Ed.), *Sea Urchins: Biology and Ecology*, 3rd ed. Academic Press, San Diego, CA, pp. 475–490.
- Weber, J.N., 1973. Temperature dependence of magnesium in echinoid and asteroid skeletal calcite: a reinterpretation of its significance. *J. Geol.* 81, 543–556.
- Whiteley, N., Mackenzie, C., 2016. Physiological responses of marine invertebrates to thermal stress. In: Sloan, M., Whiteley, N. (Eds.), *Stressors in the Marine Environment: Physiological and Ecological Responses. Societal Implications*. University Press, Oxford, pp. 56–72.
- Wolfe, K., Dworjanyn, S.A., Byrne, M., 2013. Effects of ocean warming and acidification on survival, growth and skeletal development in the early benthic juvenile sea urchin (*Helicodiaris erythrogramma*). *Glob. Chang. Biol.* 19, 2698–2707.
- Wood, H.L., Spicer, J., Lowe, D., Widdicombe, S., 2010. Interaction of ocean acidification and temperature: the high cost of survival in the brittlestar *Ophiura ophiura*. *Mar. Biol.* 157, 2001–2013.